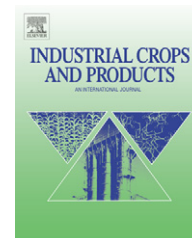


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Short communication

Ultrahigh CO₂ levels enhances cuphea growth and morphogenesis[☆]

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ABSTRACT

Applications of ultrahigh CO₂ treatments accelerated cuphea (*Cuphea viscosissima* × *C. lanceolata* ‘PSR23’) growth and development and aided in seedling establishment. The growth (fresh weight) and morphogenesis (number of leaves and roots and seedling length) were determined in cuphea seedlings exposed to 350, 1500, 3000, 10,000, or 30,000 μmol mol⁻¹ CO₂ for 30 days under greenhouse conditions. Greater CO₂ levels, especially the ultrahigh levels (i.e. ≥3000 μmol mol⁻¹ CO₂) resulted in significantly higher ($P \leq 0.05$) fresh weights, leaf numbers, root numbers, and seedling lengths compared to seedlings grown under ambient air (350 μmol mol⁻¹ CO₂). For example, cuphea ‘PSR23’ Morris heavy seedlings showed the greatest seedling fresh weight, leaf number, root number, and seedling length when supplemented with 10,000 μmol mol⁻¹ CO₂ increasing 607%, 184%, 784%, and 175%, respectively, when compared to seedlings grown without CO₂ enrichment.

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1. Introduction

Cuphea (Lythraceae) is an annual native species which produces an oil seed that is high in saturated, medium-chain triacylglycerols and can be used for making detergents, surfactants, lubricants, and other products (Cermak et al., 2005; Phippen et al., 2006). The unique seed oil composition coupled to high commercial demand suggests the possibility for future cuphea cultivation as a new crop. However, a number of negative traits retard optimum cuphea cultivation such as indeterminate flowering, seed shattering, seed dormancy, lack of self-pollination, and occurrence of sticky glandular hairs on foliage (Knapp, 1993; Isbell,

2002). Cuphea ‘PSR23’ seedlings unique among cuphea lines expresses temporary arrested growth following germination which may last from 2 to 5 weeks and hampers rapid breeding efforts. Breeding work would benefit from accelerated seedling growth, therefore, we sought to test the elevated levels of CO₂ on cuphea. Further, methods to accelerate cuphea seedling growth would also benefit tissue culture plantlet establishment in the soil and their subsequent growth. Prior studies with elevated CO₂ environments have demonstrated its ability to enhance growth with mint (Tisserat, 2002), pine (Groninger et al., 1996; Tisserat and Vaughn, 2003), potato (Schapendonk et al., 2000), soybean (Rodgers et al., 2004), and sweetgum (Groninger et al., 1996). The influence of ele-

[☆] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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vated CO₂ treatments on cuphea has not been determined to date.

Usually, CO₂ enrichment (700–1000 $\mu\text{mol mol}^{-1}$ CO₂) promotes enhanced growth of seedlings in several different species (Groninger et al., 1996; Schapendonk et al., 2000; Rodgers et al., 2004). However, it has been recently demonstrated that ultrahigh CO₂ levels ($\geq 3000 \mu\text{mol mol}^{-1}$ CO₂) enhances growth in several C-3 photosynthesis species more significantly than employing lower CO₂ levels (Tisserat, 2002; Tisserat and Vaughn, 2003). The present study was conducted to determine the growth and morphogenesis response of immature cuphea seedlings to a broad range of CO₂ levels and to determine if short CO₂ treatments may reduce seedling nursery growing time.

2. Materials and methods

2.1. CO₂ flow systems

CO₂ flow through testing chambers were made out of a transparent polycarbonate box and lid (Consolidated Plastics, Twinsburg, OH) (45.7 cm W \times 66 cm L \times 76.2 cm D; 162.6 L capacity) with a silicone tape gasket (Furon, New Haven, CT) attached. Three polypropylene spigots (Ark-Plas Products, Flippin, AR) attached to 0.45 μm air vents (Gelman Science, Ann Arbor, MI) were mounted to the box. The box and lid were clamped with 12 equally spaced stationary binding clips (50 mm L) to create an air tight seal. CO₂ was provided by a gas cylinder (BOC Gases, Edison, NJ) rated 99.8% pure and was mixed with an ambient air flow produced by an aquarium air pump (Whisper 2000, Carolina Biological Supply Company, Burlington, NC) via a flow meter (Cole Parmer Instrument Co., Niles, IL) to provide 350 (control, without CO₂ enrichment), 1500, 3000, 10,000, or 30,000 $\mu\text{mol mol}^{-1}$ CO₂. CO₂ ranges $\geq 10,000 \mu\text{mol mol}^{-1}$ CO₂ were adjusted using a LIRA infrared gas analyzer (model #3000, Mine Safety Appliances Company, Pittsburgh, PA) and CO₂ ranges $\leq 3000 \mu\text{mol mol}^{-1}$ CO₂ were adjusted with a Li-Cor CO₂/H₂O infrared gas analyzer (model LI-6262, Li-Cor, Inc., Lincoln, NE). The CO₂ and air streams were added at 2000 mL min⁻¹ during the photoperiod (14 h/day). Control seedlings were given a stream of ambient air generated by the aquarium pump only. No CO₂ or air control was applied during the dark (10 h/day).

2.2. Experiments

2.2.1. *Cuphea viscosissima* \times *C. lanceolata* L.

'PSR23' types 'McCoy GT #1', 'Morton GT #1' and 'Morris heavy' seedlings were grown in cone-tainers (Hummert Internat., Earth City, MO; 25 mm in diameter \times 160 mm in length) containing 10 g of a soilless medium formulated with peat moss:vermiculite mixed at 1:1 volume ratio and amended with 10.9 g kg⁻¹ Micromax (Scotts Co., Marysville, OH) and 62.3 g kg⁻¹ Osmocote 14-14-14 (Scotts Co.). To determine the optimum CO₂ levels for growth and morphogenesis, forty 2-week-old uniform sized cuphea seedlings were grown under 350, 1500, 3000, 10,000, or 30,000 $\mu\text{mol mol}^{-1}$ CO₂ within 162.2 L transparent containers. Seedlings were watered two times per week and not additionally fertilized during the

experimental CO₂ incubation periods. Seedlings were grown within CO₂ test chambers were maintained in a temperature-controlled greenhouse. All treatments were run concurrently and exposed to the same temperature and lighting regimes during the experiment. Experiments were repeated twice during March through June 2006. Average daily temperature was 25.2 °C and varied from 20.8 to 29.2 °C. Illumination during experiments was provided by natural sunlight with an average daily photosynthetic photon flux of 550 $\mu\text{mol m}^{-2} \text{s}^{-1}$. After 30 days of incubation, data on whole seedling fresh weight, leaf number per seedling, root number per seedling, and length per seedling were recorded from five randomly selected seedlings for each CO₂ treatment. Best fit regression equations were calculated (Table Curve.2D ver. 5.0, 2000 AISN Software, Inc.) for each response variable as a function of CO₂ treatment. Regres-

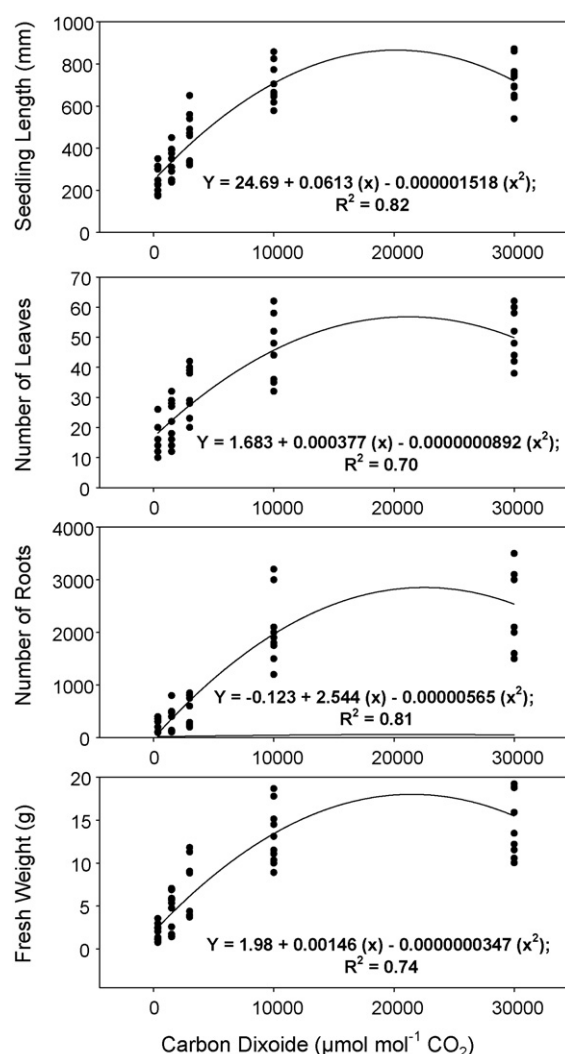


Fig. 1 – Growth and morphogenesis responses of cuphea 'PSR23' Morris heavy seedlings exposed to various concentrations of CO₂ for 30 days. Observations from two replications are presented. Regression coefficients of determination (R^2) and regression equations between CO₂ and length/seedling, leaves/seedling, roots/seedling, and fresh weight/seedling are given. All regressions were significant at $P \leq 0.05$.

Table 1 – Pearson correlation coefficients values for growth, morphogenesis and CO₂^a

	CO ₂	Leaves	Roots	Fresh weight (g)
CO ₂	–	0.743	0.816	0.755
Leaves	0.743	–	0.825	0.927
Roots	0.816	0.825	–	0.878
Fresh weight (g)	0.755	0.927	0.878	–
Seedling length (cm)	0.743	0.854	0.818	0.864

^a All values were significant at $P \leq 0.05$. Observations: leaves, 10; roots, 10; fresh weight, 10; seedling length, 10.

sion model analyses and 95% confidence limits on the mean predicted values of the response variables from the individual equations were obtained from the REG procedure of SAS (SAS Institute, ver. 8.2, Cary, NC).

3. Results and discussion

Short-term exposure (30 days) to various elevated CO₂ levels significantly affected growth and morphogenesis of seedlings for all cuphea ecotypes tested (Fig. 1). Increasing CO₂ concentrations up to 10,000 $\mu\text{mol mol}^{-1}$ CO₂ proportionally increased whole seedling fresh weight, root number, seedling length and leaf number compared to seedlings grown under ambient CO₂ levels. Smaller increases in growth and morphogenesis responses occurred in cuphea seedlings when grown under 30,000 $\mu\text{mol mol}^{-1}$ CO₂ compared to that obtained employing 10,000 $\mu\text{mol mol}^{-1}$ CO₂. Fresh weight of seedlings, leaves per seedling, roots per seedling, and seedling length in cuphea Morris heavy seedlings increased 607%, 184%, 784%, and 175%, respectively, after 30-day exposure to 10,000 $\mu\text{mol mol}^{-1}$ CO₂ over those obtained from seedlings grown on ambient CO₂ levels (Fig. 1). All cuphea seed types showed similarly response trends to increasing CO₂ concentrations (data not shown). Other investigators have found that elevated CO₂ environments (e.g., 600–800 $\mu\text{mol mol}^{-1}$ CO₂) enhanced other species fresh weights (Groninger et al., 1996; Schapendonk et al., 2000). For example, Groninger et al. (1996) found a 37% biomass increase in loblolly pine seedling when cultured under 806 $\mu\text{mol mol}^{-1}$ CO₂ after several months of exposure. In these 30-day experiments, we obtained a significant increase in biomass employing ultrahigh CO₂ (≥ 3000 $\mu\text{mol mol}^{-1}$ CO₂) levels over ambient air. There are close associations between CO₂ levels, growth and morphogenesis responses (Table 1). As CO₂ levels increase, growth and morphogenesis responses correspondingly increase. CO₂ levels had strong significant positive correlations with fresh weights, seedling length, number of

leaves and number of roots. Similarly, all growth, morphogenesis responses and CO₂ levels showed strong significant positive correlations among themselves.

Our studies were conducted for relatively short periods, i.e. 30 days, because we sought to develop a suitable short-term nursery CO₂ application treatment which would be financially and spatially practical for growing cuphea seedlings in ultrahigh CO₂ conditions within a greenhouse environment. We noted that significant differences in seedling growth were visible after 7–10 days. Ten-day-old seedlings grown in 10,000 $\mu\text{mol mol}^{-1}$ CO₂ were considerably larger than seedlings grown under ambient air. Plants removed from CO₂ chambers continued to grow normally thereafter and did not exhibit any adverse effects from their prior CO₂ treatments. Our data suggests that ultrahigh CO₂ treatments may be effective for enhancing cuphea growth and benefit breeding treatments.

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